



Seven New Drimane-Type Sesquiterpenoids from Cultures of Fungus *Phellinus tuberculosus*

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Abstract Seven new drimane-type sesquiterpenoids, phellinuins A–G (1–7), together with one known compound 3 β ,11,12-trihydroxydrimene (8) were isolated from the cultures of mushroom *Phellinus tuberculosus*. Their structures were elucidated on the basis of NMR and MS spectroscopic data and by comparison with data reported in the literature.

Keywords *Phellinus tuberculosus* · Drimane-type sesquiterpenoids · Phellinuins A–G

1 Introduction

Phellinus is a genus of fungi in the family Hymenochaetaceae. Many species cause white rot. Its fruiting bodies, often growing on wood, are resupinate, sessile, and perennial. The flesh is tough and woody or cork-like, and brown in color [1]. The fungus *Phellinus tuberculosus* has a wide distribution in Yunnan province of China [2]. The crude extract of mushroom *P. tuberculosus* was reported to possess antioxidant activity, which exhibited potent radical scavenging activity [3]. However, the chemical constituents of *P. tuberculosus* has not reported yet. As our continuous search for natural products from higher fungi [4–7], we carried out the chemical investigation on cultures of *P.*

tuberculosus, which resulted in the isolation of seven new drimane-type sesquiterpenoids named phellinuins A–G (1–7) and one known compound (8) (Fig. 1). The structures of new compounds were determined on the basis of extensive spectroscopic analysis including NMR, MS, IR data, while the known compound was identified as 3 β ,11,12-trihydroxydrimene (8) by comparison with data reported in literature [8]. This paper describes their isolation and structural elucidation.

2 Results and Discussion

Compound 1 was obtained as a colorless oil. Its molecular formula C₁₅H₂₆O₄ was revealed on the basis of the HREIMS at *m/z* 270.1827 (calcd for C₁₅H₂₆O₄, 270.1831 [M]⁺), suggesting three degrees of unsaturation. The ¹H NMR (Table 1) and ¹³C NMR data (Table 2) shows the presence of two methyls, six methylenes, four methines, and three quaternary carbons. In addition, the IR spectrum showed the presence of hydroxy group (3405 cm^{−1}). Apart from one double bond, the remaining two degrees of unsaturation in 1 were assumed to be a bicyclic sesquiterpenoid. Detailed analysis of NMR data suggested that compound 1 should be a drimane-type sesquiterpenoid with a similar planar structure to that of 3 α ,11,15-trihydroxydrimene [9]. Analysis of 2D NMR data suggested that only Me-12 was

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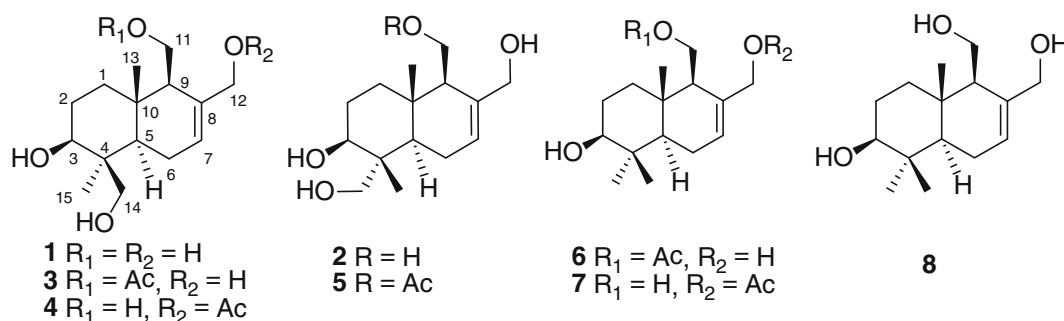


Fig. 1 Structures of compounds 1–8

Table 1 1H NMR data (600 MHz) of compounds 1–7 in methanol- d_4

No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	7 ^a
1	2.08, dt (13.8, 3.0) 1.32, overlap	2.03, overlap 1.29, td (13.8, 3.6)	2.08, overlap 1.32, overlap	2.08, overlap 1.32, overlap	2.00, m 1.27, m	2.01, m 1.29, m	2.03, m 1.29, m
2	1.80, m 1.75, m	1.66, m	1.80, m 1.75, m	1.80, m 1.75, m	1.67, m	1.63, m	1.63, m
3	3.39, dd (11.8, 3.6)	3.65, dd (11.6, 4.2)	3.39, dd (11.7, 3.6)	3.39, dd (12.0, 3.6)	3.63, dd (12.0, 4.8)	3.20, dd (13.8, 7.8)	3.20, dd (12.6, 4.8)
5	1.35, dd (12.6, 4.8)	1.63, m	1.35, dd (12.6, 4.8)	1.35, dd (12.6, 4.8)	1.63, overlap	1.25, m	1.23, dd (10.8, 4.8)
6	2.16, m 1.95, t (3.6)	2.04, overlap	2.16, m 1.97, t (3.6)	2.16, m 1.95, t (3.6)	2.03, overlap	2.08, m	2.07, m
7	5.77, t (3.0)	5.79, br. s	5.82, br. s	5.82, br. s	5.83, br. s	5.85, br. s	5.84, br. s
9	2.05, overlap	2.07, overlap	2.23, br. s	1.99, overlap	2.24, br. s	2.22, br. s	1.97, br. s
11	3.83, dd (10.8, 3.0) 3.62, dd (11.4, 7.2)	3.85, dd (10.8, 3.0) 3.62, dd (11.4, 7.2)	4.30, dd (12.0, 4.2) 4.20, overlap	3.82, dd (10.8, 4.8) 3.58, dd (10.8, 6.6)	4.32, dd (11.4, 4.2) 4.21, dd (11.4, 6.0)	4.31, dd (11.4, 4.2) 4.20, dd (11.4, 6.0)	3.82, dd (10.8, 3.6) 3.58, dd (10.8, 6.6)
12	4.23, d (11.8) 3.95, d (11.8)	4.25, d (12.6) 3.97, d (12.6)	4.05, d (12.6) 3.95, d (12.6)	4.70, d (12.6) 4.53, d (12.6)	4.06, d (12.6) 3.97, d (12.6)	4.06, d (12.6) 3.97, d (12.6)	4.70, d (12.6) 4.54, d (12.6)
13	0.79, s	0.84, s	0.84, s	0.80, s	0.90, s	0.86, s	0.82, s
14	4.19, d (11.4) 3.48, d (11.4)	0.77, s	4.19, d (11.4) 3.48, d (11.4)	4.19, d (11.4) 3.48, d (11.4)	0.80, s	0.87, s	0.86, s
15	1.20, s	3.48, d (10.8) 3.25, dd (10.8)	1.20, s	1.20, s	3.49, d (10.8) 3.25, d (10.8)	0.98, s	0.98, s
OAc			2.01, s	2.01, s	2.02, s	2.01, s	2.03, s

^a Spectra were measured at 600 MHz

^b Spectra were measured at 500 MHz

oxygenated into an oxymethylene in **1**, which was suggested by the HMBC correlations from δ_H 4.23 (1H, d, $J = 11.8$ Hz, H-12a) and 3.95 (1H, d, $J = 11.8$ Hz, H-12b) to δ_C 138.7 (s, C-8). The HMBC data further supported that the other parts of the planar structure of **1** were the same to those of 3 α ,11,15-trihydroxydrimene (Fig. 2) [9]. In the ROESY spectrum (Fig. 2), the correlation of H-5/Me-15 suggested that C-14 was β oriented, while the correlation of Me-15/H-3, as well as the constant coupling of H-3 (dd, $J = 11.8, 3.6$ Hz), indicated OH-3 to be β oriented. On the basis of these data, the ROESY correlations of H-5/H-9 and Me-13/H-11 indicated that H-9 was α oriented, while Me-13 was β oriented. Therefore,

compound **1** was established as 3 β ,11,12,14-tetrahydroxydrimene and named as phellinu A.

Compound **2** was isolated as amorphous powder. The molecular formula was established to be $C_{15}H_{26}O_4$ on the basis of the HREIMS at m/z 270.1828 (calcd for $C_{15}H_{26}O_4$, 270.1831 $[M]^+$). The 1D NMR data (Tables 1 and 2) were very similar to those of compound **1**, which indicated that both compounds had the same structure. However, the ROESY correlations of H-5/H-15 and H-15/H-3 indicated that Me-15 was oxygenated into an oxymethylene in **2**, while C-14 should be a methyl (Fig. 2). Detailed analyses of other 2D NMR data suggested that the other parts of **2** were the same to those of

Table 2 ^{13}C NMR data of compounds **1–7** in methanol- d_4

No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	7 ^a
1	38.8, CH ₂	38.6, CH ₂	38.9, CH ₂	38.8, CH ₂	38.7, CH ₂	38.9, CH ₂	39.0, CH ₂
2	28.8, CH ₂	27.8, CH ₂	28.8, CH ₂	28.7, CH ₂	27.7, CH ₂	28.6, CH ₂	28.8, CH ₂
3	81.4, CH	73.7, CH	81.3, CH	81.3, CH	73.5, CH	79.5, CH	79.7, CH
4	43.1, C	43.6, C	43.1, C	43.0, C	43.6, C	39.8, C	39.9, C
5	51.7, CH	43.0, CH	51.6, CH	51.1, CH	43.0, CH	50.7, CH	50.8, CH
6	24.4, CH ₂	24.0, CH ₂	24.4, CH ₂	24.5, CH ₂	24.0, CH ₂	24.2, CH ₂	24.5, CH ₂
7	126.2, CH	126.3, CH	126.7, CH	128.8, CH	126.7, CH	126.7, CH	129.1, CH
8	138.7, C	138.5, C	137.4, C	134.4, C	137.2, C	137.2, C	134.3, C
9	55.8, CH	55.7, CH	52.2, CH	55.7, CH	52.1, CH	52.1, CH	55.8, CH
10	36.5, C	36.4, C	36.8, C	36.6, C	36.7, C	36.9, C	36.8, C
11	61.3, CH ₂	61.4, CH ₂	63.6, CH ₂	60.9, CH ₂	63.6, CH ₂	63.5, CH ₂	60.9, CH ₂
12	66.8, CH ₂	67.0, CH ₂	65.8, CH ₂	68.6, CH ₂	65.9, CH ₂	65.7, CH ₂	68.7, CH ₂
13	16.1, CH ₃	15.7, CH ₃	16.1, CH ₃	16.0, CH ₃	15.8, CH ₃	15.1, CH ₃	15.0, CH ₃
14	65.0, CH ₂	12.6, CH ₃	65.0, CH ₂	65.0, CH ₂	12.6, CH ₃	15.9, CH ₃	16.1, CH ₃
15	23.3, CH ₃	66.6, CH ₂	23.3, CH ₃	23.3, CH ₃	66.5, CH ₂	28.0, CH ₃	28.2, CH ₃
OAc			21.2, CH ₃	21.1, CH ₃	21.2, CH ₃	21.1, CH ₃	21.1, CH ₃
OAc			173.0, C	173.0, C	173.0, C	172.9, C	173.0, C

^a Spectra were measured at 150 MHz^b Spectra were measured at 125 MHz

1. Therefore, the structure of compound **2** was established as $3\beta,11,12,15$ -tetrahydroxydrimene and named phellinuin B.

Compound **3**, a colorless oil, possessed a molecular formula $\text{C}_{17}\text{H}_{28}\text{O}_5$, as deduced from HREIMS at m/z 312.1935 (calcd for $\text{C}_{17}\text{H}_{28}\text{O}_5$, 312.1937 $[\text{M}]^+$). The IR spectrum displayed the absorption bands for $\text{C}=\text{O}$ (1722 cm^{-1}), OH (3418 cm^{-1}), and $\text{C}=\text{C}$ (1642 cm^{-1}). All the NMR data suggested that compound **3** was closely related to **1** except one more *O*-acetyl group in **3**. The *O*-acetyl group was substituted at C-11—as revealed by HMBC correlations from δ_{H} 4.30 (1H, dd, $J = 12.0$, 4.2 Hz, H-11a) and 4.20 (1H, overlap, H-11b) to δ_{C} 173.0 (s, OAc) and 52.2 (d, C-9). The other 2D NMR data suggested that the other parts of **3** were the same to those of **1** (Fig. 2). Thus, the structure of compound **3** was elucidated as phellinuin C as shown in Fig. 1.

Compound **4** was also obtained as a colorless oil. HREIMS gave one pseudomolecular ion at m/z 312.1947 (calcd for $\text{C}_{17}\text{H}_{28}\text{O}_5$, 312.1937). The 1D NMR data (Tables 1 and 2) were very similar with those of compound **3**. However, the HMBC correlations from δ_{H} 4.70 (1H, d, $J = 12.6$ Hz, H-12a) and 4.53 (1H, d, $J = 12.6$ Hz, H-12b) to δ_{C} 134.4 (s, C-8) and 173.0 (s, OAc) suggested that the *O*-acetyl group was substituted at C-12 in **4** rather than at C-11 in **3**. The other parts of structure **4** were established to be the same with those of **3** by 2D NMR correlations (Fig. 2). Therefore, compound **4** was identified as phellinuin D.

Compound **5** was established as an *O*-acetyl derivative of **2**, which was supported by the HMBC correlations from δ_{H} 4.32 (1H, dd, $J = 11.4$ and 4.2 Hz, H-11a) and 4.21 (1H, dd, $J = 11.4$ and 6.0 Hz, H-11b) to δ_{C} 137.2 (s, C-8) and 173.0 (s, OAc). The other 2D NMR data suggested that the others parts of **5** were the same to those of **2**. Therefore, the structure of compound **5** was established and named as phellinuin E.

Compounds **6** and **7** were identified as *O*-acetyl analogues of the known compound $3\beta,11,12$ -trihydroxydrimene (**8**) [8]. The HMBC data suggested that the *O*-acetyl group was substituted at C-11 in **6** and C-12 in **7**, respectively (Fig. 2). Analyses of other 2D NMR data suggested that the other parts were the same to those of **8** (Fig. 2). Therefore, the structures of compounds **6** and **7** were established and named as phellinuin F (**6**) and phellinuin G (**7**), respectively.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured on a Jasco-P-1020 polarimeter. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instruments of Avance III 600 and Bruker DRX-500. HREIMS were measured on a waters autoSpec Premier P776 instruments. Silica gel (200–300

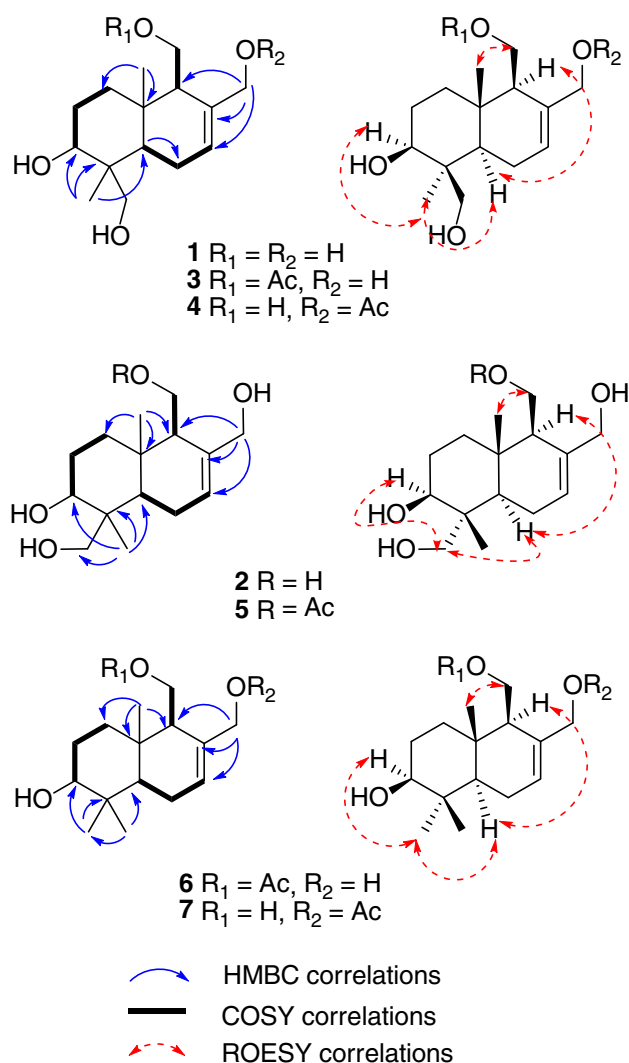


Fig. 2 The main HMBC, COSY, and ROESY correlations of 1–7

mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC and spots were visualized by heating silica gel plates immersed in vanillin- H_2SO_4 in EtOH, in combination with Agilent 1200 series HPLC system (Eclipse XDB-C18 column, 5 μm , 4.6 \times 150 mm).

3.2 Material and Cultural Conditions

Fruiting bodies of *Phellinus tuberculosus* were collected at Jingdong, Yunnan Province, China in 2003 and identified by Prof. Zhu-Liang Yang of Kunming Institute of Botany, CAS. The voucher specimen (NO.CGBWSHF00118) was deposited at herbarium of Kunming Institute of Botany. Culture medium: glucose (5 %), pork peptone (0.15 %), yeast (0.5 %), KH_2PO_4 (0.05 %), $MgSO_4$ (0.05 %). The initial pH was adjusted to 6.0, the fermentation was first

carried out on an Erlenmeyer flask for 6 days till the mycelium biomass reached to the maximum. Later it was transferred to a fermentation tank (20 L) at 24 $^{\circ}C$ and 250 rpm for 20 days, ventilation was set to 1.0 vvm (vvm: air volume/culture volume/min).

3.3 Extraction and Isolation

The culture broth (20 L) was concentrated under vacuum, extracted three times with EtOAc. The organic layer was evaporated in vacuum to give a crude extract (3.1 g), which was separated by Sephadex LH-20 (MeOH) CC to afford fractions A–C. Fraction B (2.5 g) was separated by reversed-phased C18 column (MeOH- H_2O , 30–100 %) to give sub-fractions B1 and B5. The sub-fraction B1 (110 mg) was further purified by silica gel ($CHCl_3$ -MeOH, 20/1) to yield 1 (5.8 mg), Fraction B2 was separated by Sephadex LH-20 (MeOH) to obtain B2-1 (50 mg), which was further isolated and purified by silica gel CC ($CHCl_3$ -MeOH, 20/1) to obtain 2 (31.6 mg). Fraction B3 was purified by Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$ -MeOH, 30/1) to get 5 (2.2 mg) and 8 (8.6 mg). The sub-fraction B4 was purified by preparative HPLC (MeOH- H_2O , 40 %, 10 mL/min) to obtain 3 (7.9 mg) and 4 (2.5 mg). Fraction B5 was separated by Sephadex LH-20 (MeOH) column chromatography, then further separated on silica gel ($CHCl_3$ -MeOH, 30/1) to give 6 (17.4 mg) and 7 (1.0 mg).

Phellinuin A (1): colorless oils, $[\alpha]_D^{17.3} - 10.3$ (c 0.1 MeOH); IR (KBr) ν_{max} 3405, 2963, 2930, 2871, 1665, 1443 cm^{-1} ; 1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (neg.) m/z 539 $[2M - H]^-$; HREIMS m/z 270.1827 $[M]^+$ (calcd for $C_{15}H_{26}O_4$, 270.1831).

Phellinuin B (2): amorphous powder, $[\alpha]_D^{20.6} 0$ (c 0.3 MeOH); IR (KBr) ν_{max} 3345, 2930, 2878, 1666, 1442, 1057, 1983 cm^{-1} ; 1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 539 $[2M - H]^+$; HREIMS m/z 270.1828 $[M]^+$ (calcd for $C_{15}H_{26}O_4$, 270.1831).

Phellinuin C (3): colorless oil, $[\alpha]_D^{20.6} - 4.2$ (c 0.2 MeOH); IR (KBr) ν_{max} 3418, 2966, 2933, 2859, 1722, 1642, 1454, 1385, 1256 cm^{-1} ; 1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 335 $[M + Na]^+$; HREIMS m/z 312.1935 $[M]^+$ (calcd for $C_{17}H_{28}O_5$, 312.1937).

Phellinuin D (4): colorless oil, $[\alpha]_D^{20.8} - 5.4$ (c 0.3 MeOH); IR (KBr) ν_{max} 3421, 2961, 2932, 2858, 1736, 1631, 1443, 1384, 1254 cm^{-1} ; 1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 335 $[M + Na]^+$; HREIMS m/z 312.1947 $[M]^+$ (calcd for $C_{17}H_{28}O_5$, 312.1937).

Phellinuin E (5): colorless oil, $[\alpha]_D^{21.1} + 5.6$ (c 0.002 MeOH); IR (KBr) ν_{max} 3424, 2932, 2891, 1736, 1634, 1456 cm^{-1} ; 1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 335 $[M + Na]^+$; HREIMS m/z 312.1930 $[M]^+$ (calcd for $C_{17}H_{28}O_5$, 312.1937).

Phellinuin F (6): colorless oil, $[\alpha]_D^{20.4} - 2.8$ (c 0.4 MeOH); IR (KBr) ν_{\max} 3441, 2965, 2931, 2857, 1737, 1639, 1442 cm^{-1} ; ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 319 $[\text{M} + \text{Na}]^+$; HREIMS m/z 296.1986 $[\text{M}]^+$ (calcd for $\text{C}_{17}\text{H}_{28}\text{O}_4$, 296.1988).

Phellinuin G (7): colorless oil, $[\alpha]_D^{20.4} - 6.5$ (c 0.1 MeOH); IR (KBr) ν_{\max} 3442, 2933, 2869, 1721, 1665, 1460, 1254 cm^{-1} ; ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); ESIMS (pos.) m/z 4319 $[\text{M} + \text{Na}]^+$; HREIMS m/z 296.1982 $[\text{M}]^+$ (calcd for $\text{C}_{17}\text{H}_{28}\text{O}_4$, 296.1988).

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